

Mitochondrial Mix

Combined Approach to Visualizing Oxidative Stress in Real Time

Oxidative stress resulting from mitochondrial dysfunction may play a role in toxicity caused by many different environmental contaminants, including aromatic hydrocarbons and metal ions, but it has been difficult to evaluate the role of mitochondria in oxidative stress using currently available methods. A new study combining new and established imaging techniques to document mitochondrial dysfunction now indicates this integrated approach to real-time imaging in live cells can be useful for studying the role of oxidative stress in toxicologic responses [*EHP* 118(7):902–908; Cheng et al.].

Real-time imaging in live cells to detect products of oxidative stress known as reactive oxygen species (ROS) offers superior temporal and spatial resolution compared with traditional methods such as detecting oxidized lipids, proteins, or DNA. But the accuracy and utility of the fluorescent indicator H2DCF-DA, a reagent commonly used for directly detecting ROS in living cells, is limited.

The authors conducted a set of experiments in which they exposed cultured human skin carcinoma cells to zinc (Zn^{2+}), a ubiquitous contaminant known to induce oxidative stress. Three different fluorescent imaging techniques were used to study effects of Zn^{2+} on mitochondria. The first used the fluorophore PG1 to measure production of the ROS hydrogen peroxide. The scientists found that hydrogen peroxide increased within the cells upon Zn^{2+} exposure and that its production was inhibited with the addition of the mitochondrial

inhibitor CCCP, implicating mitochondria as the source of the Zn^{2+} -induced hydrogen peroxide.

A second experiment used the fluorescent indicator JC-1 to measure changes in mitochondrial membrane potential (the difference in electrical potential between the inside and outside of the mitochondrial membrane) following exposure to Zn^{2+} . When Zn^{2+} was administered, loss of JC-1 fluorescence emission indicated a loss of membrane potential consistent with impaired mitochondrial function.

A third experiment used the genetically encoded fluorescent sensor MTroGFP1 to measure the redox potential of mitochondria after Zn^{2+} exposure. MTroGFP1 associates with mitochondria in transfected cells, causing the mitochondria to fluoresce. However, the investigators observed a change in the fluorescent signals following exposure to Zn^{2+} consistent with a loss of redox potential. In a fourth experiment on mitochondria isolated from live mouse hearts, the authors demonstrated that administering Zn^{2+} resulted in rapid mitochondrial swelling, another indication of a loss of mitochondrial function.

The study shows the value of combining multiple imaging techniques to constitute an integrated approach that permits real-time monitoring of the mechanisms behind oxidative stress within living cells. The results also add to the evidence that oxidative stress induced by Zn^{2+} originates in mitochondria and sheds light on some of the mechanisms that may be involved. Further study is under way to determine the exact sequence of cellular events by which toxicants induce generation of ROS and mitochondrial dysfunction.

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Endotoxin from Biomass Burning

An Underestimated Health Hazard?

Approximately 3 billion people worldwide burn biomass—wood, charcoal, dried animal dung, and crop residue—to heat their homes and cook their food. Biomass often is burned in small, poorly ventilated areas; the resulting smoke exposure frequently causes respiratory infections, primarily among women and children younger than 5 years, who spend the most time around the home fires. Recent findings suggest airborne endotoxin generated from burning biomass may play an important role in the health effects associated with biomass smoke [*EHP* 118(7):988–991; Semple et al.].

According to the World Health Organization, exposure to smoke from biomass burning is responsible for 1.5 million premature deaths annually. Previous research has focused primarily on the mass of airborne fine particulate matter as being responsible for the morbidity and mortality caused by biomass burning. These particles can penetrate deep into the lungs, causing inflammation and both acute and chronic airway and lung damage.

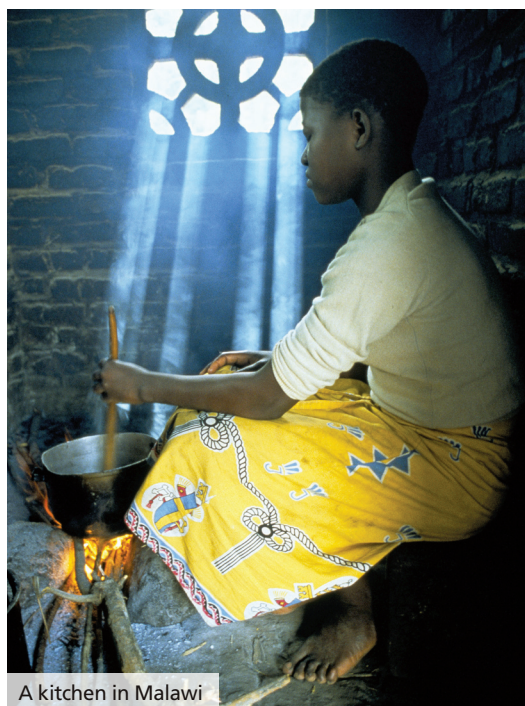
Endotoxins are part of the cell wall of gram-negative bacteria and are found in organic material. These molecules can

cause lung inflammation and have previously been found in tobacco smoke and in homes where there are pets and mold.

To evaluate the presence of airborne endotoxin in homes where biomass is burned, the researchers set up air sampling monitors in 31 homes in Nepal and 38 homes in Malawi. Average levels of inhalable endotoxin measured over 24 hours in Malawian homes were 24 endotoxin units (EU)/m³ for charcoal-burning homes and 40 EU/m³ for wood-burning homes. In Nepal, short-term measurements during cooking indicated average inhalable endotoxin levels of 365 EU/m³ for dung-burning homes and 43 EU/m³ for wood-burning homes. These figures are considerably higher than levels shown to be associated with respiratory ailments during the first two years of life in a separate study [*EHP* 114(4):610–614 (2006)].

The authors acknowledge weaknesses in their study, such as the large time gap between collection of the filters used to trap endotoxins and their analysis, which could have led to high levels of contamination on some of the materials used for collection. Despite the lack of resolution about how biomass smoke contributes to respiratory disease, write the authors, the very fact that it does so makes the use of more efficient stoves and better ventilation in homes where biomass is burned “a matter of urgency.”

Harvey Black of Madison, WI, has written for *EHP* since 1994. His work has also appeared in *Environmental Science & Technology*, *ChemMatters*, and the *Milwaukee Journal Sentinel*.



A kitchen in Malawi